

Matrix metalloproteinases in the brain and blood–brain barrier: Versatile breakers and makers

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Abstract

Matrix metalloproteinases are versatile endopeptidases with many different functions in the body in health and disease. In the brain, matrix metalloproteinases are critical for tissue formation, neuronal network remodeling, and blood–brain barrier integrity. Many reviews have been published on matrix metalloproteinases before, most of which focus on the two best studied matrix metalloproteinases, the gelatinases MMP-2 and MMP-9, and their role in one or two diseases. In this review, we provide a broad overview of the role various matrix metalloproteinases play in brain disorders. We summarize and review current knowledge and understanding of matrix metalloproteinases in the brain and at the blood–brain barrier in neuroinflammation, multiple sclerosis, cerebral aneurysms, stroke, epilepsy, Alzheimer's disease, Parkinson's disease, and brain cancer. We discuss the detrimental effects matrix metalloproteinases can have in these conditions, contributing to blood–brain barrier leakage, neuroinflammation, neurotoxicity, demyelination, tumor angiogenesis, and cancer metastasis. We also discuss the beneficial role matrix metalloproteinases can play in neuroprotection and anti-inflammation. Finally, we address matrix metalloproteinases as potential therapeutic targets. Together, in this comprehensive review, we summarize current understanding and knowledge of matrix metalloproteinases in the brain and at the blood–brain barrier in brain disorders.

Keywords

Blood–brain barrier, inflammation, multiple sclerosis, intracranial aneurysm, stroke, epilepsy, Alzheimer's, Parkinson's disease, neurodegeneration

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Introduction

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-endopeptidases of the metzincin superfamily.¹ Structurally, MMPs contain a conserved Zn²⁺-binding motif in the catalytic domain and several conserved protein domains (Figure 1).² MMPs are expressed as inactive zymogens with a pro-peptide domain (pro-MMPs) that must be removed for MMP activation. The pro-peptide is part of the “cysteine switch,” which is an intramolecular complex between a single cysteine in the pro-peptide domain and the zinc in the active site. Through cleavage of the pro-peptide, the cysteine dissociates from the complex, which activates the MMP enzyme and allows binding and cleavage of MMP substrates. MMPs also contain an amino-terminal signal sequence directing the peptide

to the endoplasmic reticulum. In addition, all MMPs, except MMP-7 and MMP-26, have a hemopexin-like domain that is connected to the catalytic domain and is responsible for MMP interactions with substrates, endogenous inhibitors, and cell-surface molecules.

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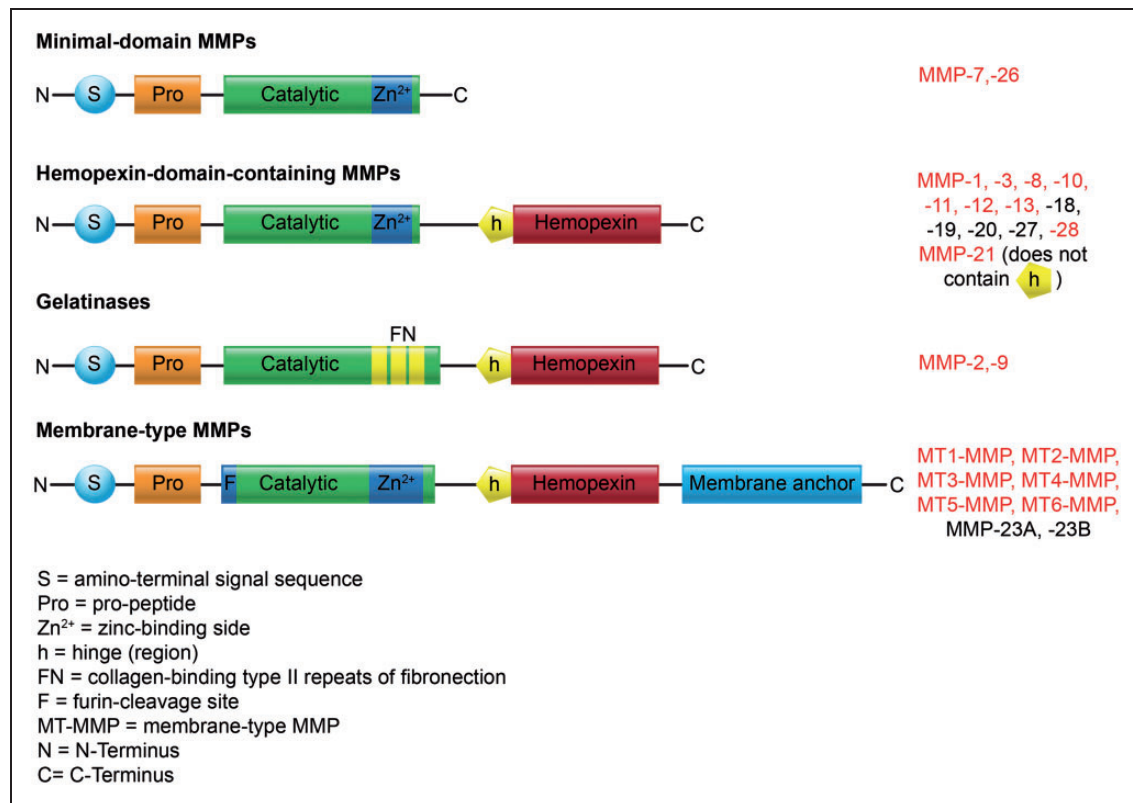


Figure 1. Matrix metalloproteinase structure. MMPs are divided into distinct structural groups: minimal-domain MMPs, hemopexin-domain-containing MMPs, gelatinases, and membrane-type MMPs. Minimal-domain MMPs contain an amino-terminal signal sequence (S) that directs them to the endoplasmic reticulum, a pro-peptide (Pro) that maintains them as inactive zymogens, and a catalytic domain with a zinc-binding site (Zn²⁺). In addition to the domains found in the minimal domain MMPs, hemopexin-domain-containing MMPs have a hemopexin-like domain that is connected to the catalytic domain via a hinge (h). This hinge region mediates the interactions with substrates, TIMPs, and cell-surface molecules. Gelatinase-type MMPs contain inserts that resemble collagen-binding type II repeats of fibronectin (FN). Membrane-type MMPs (MT-MMPs) have a domain that interacts with the membrane. Some MMPs also have a furin-cleavage site (F). MMPs found in the brain are highlighted in red.

History

The first MMP (MMP-1) was identified by Jerome Gross and Charles Lapiere in 1962 in tadpole.³ In 1968, the first human MMP was discovered in skin tissue.⁴ Since then, a large family of MMPs has been described in various species.¹ In 1971, MMPs were shown to be biosynthesized as inactive precursors (zymogens) that require activation.⁵ The first endogenous MMP inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), was identified in 1975 and as of today, four TIMPs (TIMP-1-4) have been described.^{6,7} In 1990, the “cysteine switch” MMP activation mechanism was discovered.⁸ Since then our understanding of MMP biology has increased tremendously. Through the unraveling of the MMP catalytic cycle, we now know that MMPs also digest non-extracellular matrix proteins and contribute to the fine-tuning of cellular processes. In addition, new MMPs – MMP-20, MMP-26, and MMP-28 – have been identified over the last 25 years.^{9–11}

Classification

Currently, 24 human MMP homologues have been described and are categorized into six sub-families: collagenases (MMPs-1, -8, and -13), gelatinases (MMPs-2 and -9), stromelysins (MMPs-3, -10, and -11), matrilysins (MMPs-7 and -26), membrane-type metalloproteinases (MT1-6-MMPs, also referred to as MMPs-14, -15, -16, -17, -24, and -25), and other MMPs (MMPs-12, -18, -19, -20, -21, -22, -23, -27, and -28; Table 1).^{12–14}

Function and role

MMPs play a role in normal physiological processes including tissue morphogenesis, cell migration, and angiogenesis. MMPs are also involved in pathophysiological processes including wound healing, inflammation, and cancer. Some speculate that MMPs cleave extracellular matrix (ECM) proteins to allow infiltrating cells, including leukocytes, metastatic, and

Table 1. MMPs in CNS disease.

Nomenclature	Class/trivial or alternative name	Involvement in CNS diseases
MMP-1	Collagenase-1/interstitial collagenase	Neuroinflammation ^b , epilepsy ^a , AD ^a , brain cancer
MMP-2	Gelatinase A/72 kDa gelatinase	Neuroinflammation, MS, cerebral aneurysms, stroke, epilepsy ^a , AD ^a , PD ^a , brain cancer
MMP-3	Stromelysin-1	Neuroinflammation, MS, cerebral aneurysms, stroke, epilepsy ^a , AD ^a , PD, brain cancer
MMP-7	Matrilysin-1/PUMP-1	Neuroinflammation ^b , stroke, epilepsy ^a , AD ^a
MMP-8	Collagenase-2/neutrophil collagenase	Neuroinflammation, brain cancer ^b
MMP-9	Gelatinase B/92 kDa gelatinase	Neuroinflammation, MS, cerebral aneurysms, stroke, epilepsy, AD ^a , PD ^a , brain cancer
MMP-10	Stromelysin-2	Stroke, epilepsy ^b , brain cancer
MMP-11	Stromelysin-3	MS, stroke, brain cancer
MMP-12	Macrophage metalloelastase	Neuroinflammation, MS, AD ^b
MMP-13	Collagenase-3	Neuroinflammation, brain cancer
MMP-14	Membrane-type-1 MMP (MT1-MMP)	Neuroinflammation, cerebral aneurysms, MS, PD ^a , brain cancer
MMP-15	Membrane-type-2 MMP (MT2-MMP)	MS, brain cancer
MMP-16	Membrane-type-3 MMP (MT3-MMP)	MS, brain cancer
MMP-17	Membrane-type-4 MMP (MT4-MMP)	Neuroinflammation ^a , MS ^a , AD ^b , brain cancer ^a
MMP-18	Collagenase 4, α Col4, Xenopus Collagenase	
MMP-19	RASI-1 (Stromelysin-4)	Brain cancer ^b
MMP-20	Enamelysin	
MMP-21	X-MMP	MS ^a
MMP-23	CA-MMP	
MMP-24	Membrane-type-5 MMP (MT5-MMP)	MS ^a , brain cancer ^a
MMP-25	Membrane-type-5 MMP (MT6-MMP)	MS ^a , brain cancer ^a
MMP-26	Matrilysin-2, Endometase	Brain cancer ^b
MMP-27	MMP-22, C-MMP	
MMP-28	Epilysin	MS ^a

^aMMP mRNA, protein and/or activity levels are elevated but involvement in disease is unclear.

^bLimited literature (one to two publications) available to support MMP involvement in the disorders.

transformed cells, to penetrate ECM barriers.^{14,15} However, this is controversial since the first *in vitro* studies that associated MMPs with the cleavage of ECM molecules were based on experiments using excessive amounts of MMPs. Today, evidence suggests that ECM cleavage is not a main function of MMPs *in vivo*.¹⁶

Studies using mass spectrometry indicate that ECM molecules are MMP substrates and other studies show that blocking MMPs (e.g., MT1-MMP) prevents leukocytes from crossing artificial collagen and ECM layers.^{15,17,18} In these studies, it was demonstrated that fibroblasts and tumor cells tunnel through dense barriers of cross-linked type I collagen *in vitro* or *in vivo* via a virtually indistinguishable proteolytic process that requires MMPs. Furthermore, Ota et al.¹⁹ showed that cancer cells utilize an MT1- and MT2-MMP-dependent basement membrane transmigration program to intravasate into the vasculature *in vivo*.

Other studies using MMP knockout (KO) mice and novel mass spectrometry techniques that allowed improved tissue analysis revealed a wide MMP substrate spectrum that includes cell surface molecules and soluble factors such as chemokines, cytokines, and cytokine receptors.¹⁸ MMP-mediated cleavage of these substrates modulates their activity and represents an important mechanism of fine tuning cellular processes such as inflammation.^{18,20–22} In essence, MMPs are critical for remodeling processes in developing and regenerating tissues.^{15,18}

Expression, activation, and regulation

All MMPs, with the exception of MMP-28, are ubiquitously expressed in mammalian organisms. MMP expression levels are generally low and only increase when needed,²³ except MMP-2 and MT1-MMP (and to a lesser extent MMP-9), which are constitutively

expressed in the brain in both their pro- and activated forms.^{24,25} MMPs are generated and then secreted into the extracellular space in an inactive, latent pro-form (zymogen), which is activated through proteolysis of the N-terminal pro-domain (Figure 1). This process allows rapid regulation of MMP activity, and thus, controls the availability of cytokines and chemokines. Consequently, MMPs are pivotal in controlling fast cellular responses, such as cell migration during inflammation.

Under physiological conditions, most MMPs are activated by other MMPs or proteases in the extracellular space, but some MMPs are activated intracellularly by the enzyme furin, or by other mechanisms (e.g., phosphorylation). MMP inhibition, on the other hand, is mediated by tissue inhibitors of metalloproteinases, TIMPs, that coexist with MMPs.^{7,26} TIMPs inactivate MMP activity by binding to them, which under physiological conditions prevents excessive tissue degradation and injury. Under pathophysiological conditions, MMPs can be activated by reactive oxygen species and other factors (e.g., NO, hypoxia, pH) through a mechanism that likely involves auto-catalytic activation.^{27–29}

Little is known about transcriptional MMP regulation and what is known involves inflammatory signaling. Tumor necrosis factor- α (TNF- α) and interleukin-17 both stimulate MMP-9 transcription through the transcription factors activator protein-1 (AP-1) and nuclear factor- κ B (NF- κ B).^{30–32} This effect can be blocked with interferon- γ through NF- κ B inhibition.³³ The endotoxin lipopolysaccharide (LPS) triggers reactive oxygen species production and p38 kinase phosphorylation, which activates AP-1 and induces MMP-9 transcription.³⁴ Figure 2 shows the MMP-9 promoter with one NF- κ B and two AP-1 binding sites. MMP-2, on the other hand, is regulated by TNF- α and p38-MAPK acting through NF- κ B, but

not AP-1 (Figure 2), and a caspase 8-dependent pathway.³⁵ Together, MMP regulation is not fully understood and varies between cell types in a context-dependent manner.^{36,37}

MMPs in brain and blood–brain barrier

MMPs participate in many physiological and pathological processes in the brain and at the blood–brain barrier (Table 2). The blood–brain barrier is the capillary endothelium that separates blood from brain.⁴⁰ Physical barrier function is localized to three structures that are critical for barrier integrity: the brain capillary endothelium, the tight junctions between the endothelial cells, and the basement membrane (Figure 3).⁴⁰ First, the brain capillary endothelium is a barrier for small hydrophilic compounds. Second, tight junctions seal the clefts between adjacent endothelial cells, which prevents uncontrolled paracellular passage of solutes and makes the brain endothelium a low-permeability barrier.^{41–43} The major tight junction proteins in the brain endothelium are claudin-1, claudin-5, occludin, and zonula occludens-1. Third, the basement membrane, which is a specialized ECM, connects endothelial cells with pericytes and astrocytes to form the neurovascular unit and facilitates communication between the cells within this unit through receptors such as integrins and dystroglycans.^{44,45}

Endothelial cells, tight junctions, and basement membrane are critical for proper barrier function, and thus, for brain homeostasis and overall brain health. In turn, pathological disruption of the endothelium, tight junctions, or the basement membrane impairs barrier integrity, which can have severe consequences for the brain and contribute to disease progression.^{97,98} In this regard, it has been proposed that MMPs digest tight junctions and basement membrane proteins, and thus,

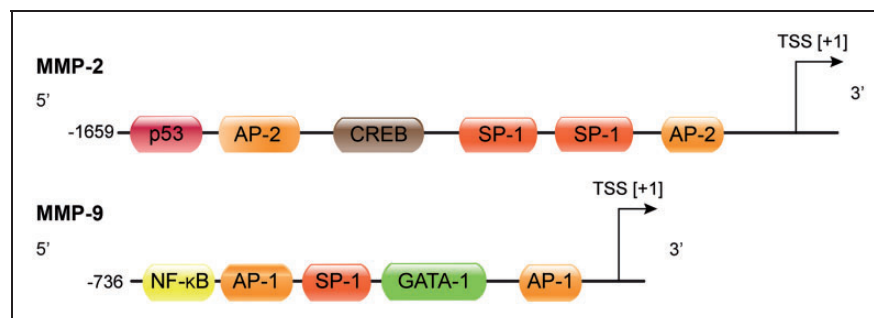


Figure 2. MMP-2 and MMP-9 promoter region with putative transcription factor binding sites. The boxes represent binding sites for the corresponding transcription factors.

TSS: transcription start site; AP-1: activator protein 1; AP-2: activator protein 2; GATA-1: GATA-binding factor 1, erythroid transcription factor, globin transcription factor 1; SP-1: specificity protein 1; NF- κ B: nuclear factor- κ B, CREB: cyclic AMP response-element binding protein; p53: tumor protein p53 (modified after Peters et al.³⁸ and Rosenberg³⁹).

are critical contributors to brain disease and directly affect brain health.^{99,100} However, little data exist to support this since it is technically challenging to demonstrate MMP activity *in vivo*. For example, Gu et al.¹⁰¹ show increased MMP activity and higher

permeability at the blood–brain barrier in stroke during reperfusion *in vivo*. Yang et al.¹⁰² show increased MMP-2 and MMP-9 mRNA and activity levels after reperfusion in spontaneously hypertensive rats with middle cerebral artery occlusion (MCAO). The authors also observed blood–brain barrier leakage in the piriform cortex and disrupted tight junction proteins. Inhibiting MMPs prevented the loss of tight junction proteins, indicating that MMPs disturb barrier integrity by degrading tight junction proteins.¹⁰² Thus, while technically challenging, first evidence showing that MMPs digest tight junctions and ECM proteins *in vivo* is emerging.

Table 2. MMP effects in the brain and blood–brain barrier.

MMPs in physiological processes	MMPs in pathophysiological processes
CNS development ^{46,47}	Involvement in CNS injuries and diseases ^{48–52,53}
CNS survival ⁵⁴	Blood–brain barrier leakage ^{55–57}
Angiogenesis ⁵⁸	Neuroinflammation ^{59–65}
Neurogenesis ^{66,67}	Neurotoxicity ^{68–77}
Cell fate determination ⁷⁸	Demyelination ^{79–82}
Proliferation and migration of cell precursors ^{17,83}	Tumor angiogenesis ^{58,84–87}
Axonal growth and regeneration ^{88–91}	Tumor metastasis ^{82,93}
Myelinogenesis and remyelination ^{47,94}	
Terminate neuroinflammation ^{95,96}	

Methods and techniques to study MMPs

MMPs are mostly studied at the mRNA, protein, and activity levels. MMP mRNA expression has been demonstrated in several studies using real-time quantitative PCR and microarray analysis.^{103,104} MMP protein expression is usually determined by Western blotting, ELISA, or by immunohistochemistry. To detect MMP activity *in vitro*, a widely used technique – substrate zymography – is employed. Substrate zymography identifies MMPs by the degradation of their substrate and by their molecular weight.^{105,106} This method allows determining if the MMP is active or

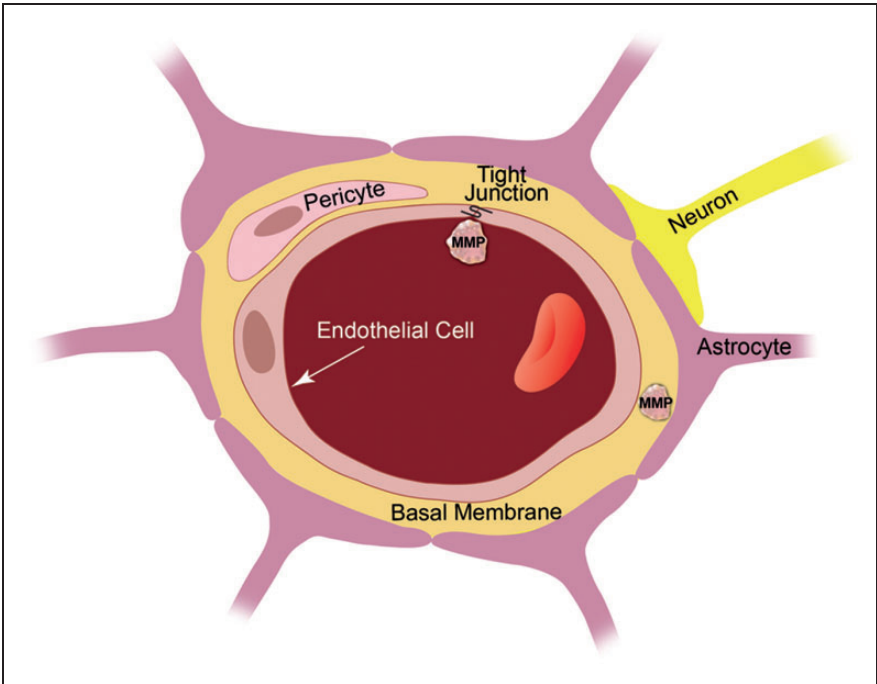


Figure 3. Blood–brain barrier anatomy. The blood–brain barrier is formed by capillary endothelial cells that are linked by tight junctions, surrounded by a basement membrane, and astrocytic endfeet. Astrocytes provide the cellular link to neurons; pericytes are embedded in the basement membrane. In disease, MMP protein expression and activity levels are increased, which is thought to result in blood–brain barrier leakage, possibly through degradation of tight junction and basement membrane proteins.

latent. All types of substrate zymography originate from gelatin zymography, which is used to detect the gelatinases MMP-2 and MMP-9.¹⁰⁷ To detect other MMPs, gelatin is replaced with another substrate such as collagen, carboxymethylated transferrin, or casein.^{104,108–111} Another method to detect MMP activity *in vitro* is by using fluorogenic MMP substrates. These artificial MMP substrates are composed of a fluorescent dye that is connected via a peptide to a quencher. Active MMP cleaves the peptide, and thus, removes the quencher resulting in fluorescence, which is a direct measure of MMP activity.¹¹²

Currently, it is not possible to localize activity of most MMPs in tissues due to a lack of suitable reagents. An exception to this is gelatin *in situ* zymography, a method that allows detecting MMP-2 and MMP-9. Gelatin *in situ* zymography is a modification of substrate zymography in frozen tissue sections. In this method, an MMP substrate is transferred onto a frozen section of an unfixed tissue sample. The substrate is digested by active MMPs in a time- and dose-dependent manner, which is visualized with microscopy.¹¹³ A further development of this method is *in vivo* zymography,¹¹⁴ where substrates are used in a live animal to detect MMP activity *in vivo*.¹¹⁵

MMPs in central nervous system disease

Neuroinflammation

Neuroinflammation is defined as an unspecific inflammatory event in the brain. All central nervous system (CNS) disorders discussed here – multiple sclerosis (MS), cerebral aneurysm, stroke, epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), and brain cancer – have a neuroinflammatory component that involves MMPs.

MMPs contribute to neuroinflammation through four mechanisms (Figure 4). First, MMPs activate neuroinflammatory pathways. This is done indirectly by activating enzymes that act on signaling molecules such as cytokines, cell surface receptors, cell–cell adhesion molecules, or clotting factors.^{59,60} Alternatively, MMPs directly activate neuroinflammatory pathways. For example, MT4-MMP has TNF- α -convertase activity through which transmembrane TNF- α is proteolytically converted into soluble, active TNF- α (Figure 4(1)).^{61,62} Second, MMPs themselves act as neuroinflammatory signaling molecules. Upon stimulation with LPS, apoptotic signals, or in PD, neurons secrete active MMP-3 into the interstitium, which triggers microglial activation and production and secretion of pro-inflammatory cytokines (Figure 4(2)).^{63–65}

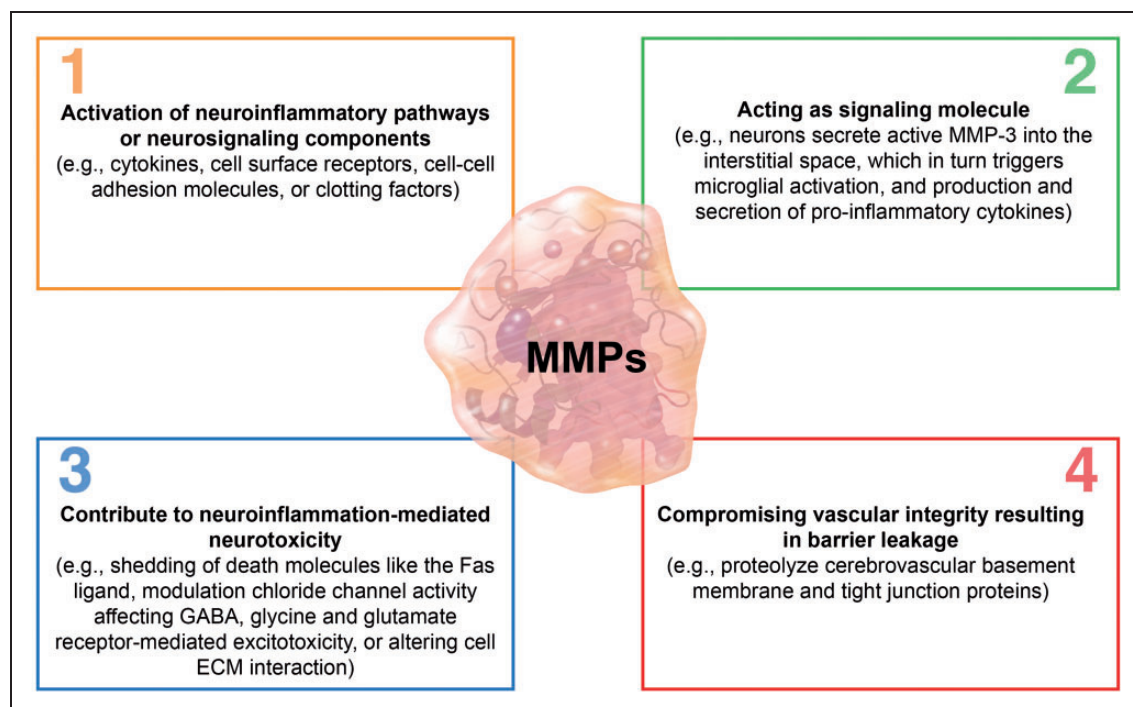


Figure 4. MMPs in neuroinflammation. MMPs contribute to neuroinflammation through four mechanisms. (1) MMPs activate neuroinflammatory pathways and/or neurosignaling components. (2) MMPs act as signaling molecules themselves. (3) MMPs contribute to neuroinflammation-mediated neurotoxicity. (4) MMPs compromise vascular integrity resulting in blood–brain barrier leakage.

Third, MMPs contribute to neuroinflammation-mediated neurotoxicity by shedding death molecules like the Fas ligand, by affecting GABA and glycine levels, which modulate chloride channel activity, by stimulating glutamate receptor-mediated excitotoxicity, or by altering cell–ECM interaction.^{68–72} However, the exact mechanisms through which MMPs exert neurotoxicity are not fully understood (Figure 4(3)).^{73–77} And lastly, neuroinflammation-induced MMPs may proteolyze cerebrovascular basement membrane and tight junction proteins, which could compromise vascular integrity resulting in barrier leakage and extravasation (Figure 4(4)).^{55–57,79}

Together, MMPs are induced by and contribute to neuroinflammation through various mechanisms. MMPs also contribute to inflammation-induced barrier dysfunction and leakage. The combination of both – neuroinflammation and barrier dysfunction – promotes progression of several CNS disorders (MS, cerebral

aneurysms, stroke, epilepsy, AD, PD, and brain cancer), which are discussed in the following sections.

Multiple sclerosis

MS is a neuroinflammatory auto-immune disease that affects about 1.3 million people worldwide.¹¹⁶ In MS, the myelin sheaths that cover neuronal axons and nerve fibers in the brain and spinal cord are damaged, which disrupts communication and causes a wide range of disease symptoms.¹¹⁷

The role of MMPs in MS has been studied extensively in animal models and human tissue.^{118–122} These studies revealed that MMPs digest myelin basic protein, which causes demyelination and drives MS progression (Figure 5(a)).^{79,81,82} Using the experimental autoimmune encephalomyelitis (EAE) animal model of MS, several groups analyzed MMPs in brain, brain capillary endothelial cells, spinal cord, lymph nodes,

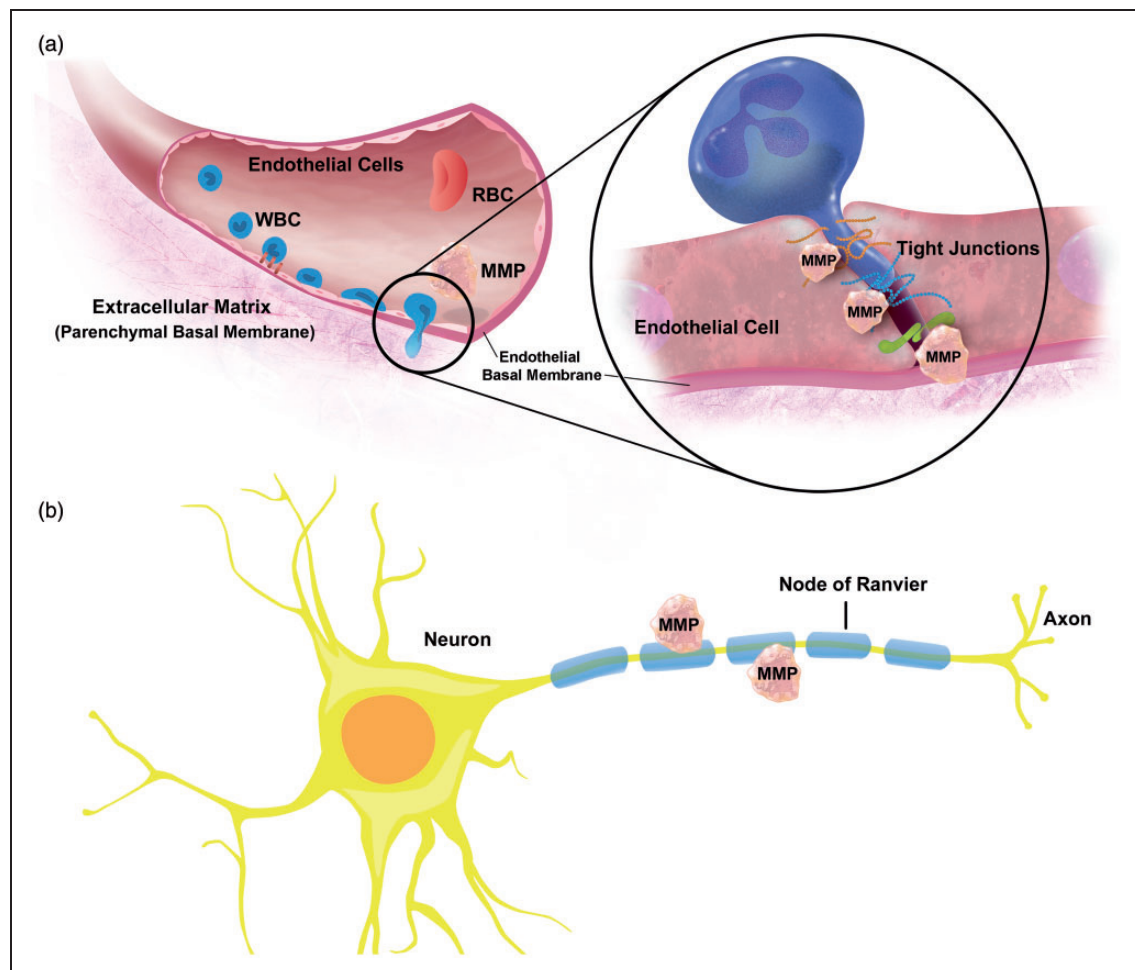


Figure 5. MMPs in multiple sclerosis. (a) Brain endothelial cells and leukocytes secrete MMPs, which are thought to degrade tight junction and extracellular matrix proteins leading to extravasation of immune cells. (b) Leukocytes, microglia, neurons, and reactive astrocytes secrete MMPs, which demyelinate neuronal axons.

and spleen and showed that multiple MMPs were elevated during the EAE peak stage.^{119,123–128} Specifically, in EAE mouse and rat models, mRNA, and protein levels were increased for MMPs-2, -3, -7, -8, -9, -10, -11, -12, -13, -28, MT1-MMP, and MT6-MMP. In contrast, mRNA and protein levels for MT2-5-MMP and MMP-21 were decreased in lumbar and sacral spinal cord tissue of EAE mice.¹²⁹ While the consequence of decreased MMP levels, particularly those of MT-MMPs, is unclear, it is well established that increased MMP levels aggravate disease severity in EAE rodent models.^{118,119,125,127,128,130,131}

One characteristic of MS is leukocyte extravasation and transmigration across the brain endothelium into the CNS. MMPs may facilitate this process by activating adhesion molecules and degrading the basement membrane that surrounds blood vessels (Figure 4(1) and (4)). However, this is controversial since there is no conclusive evidence. Agrawal et al.¹¹⁸ show selective MMP-2- and MMP-9-mediated cleavage of dystroglycan, which is a linker between astrocyte endfeet and parenchymal basement membrane molecules. This process is located at postcapillary venules, where extravasation occurs.¹¹⁸ But this and other studies, for example, the study by Buhler et al.¹¹⁹ suggest that MMPs are involved in immune cell extravasation into the brain during EAE.

Studies using brain tissue, serum, and cerebrospinal fluid (CSF) samples from MS patients consistently found increased protein levels for MMPs-2, -3, -7, -9, -12, -13, and MT1-MMP.^{80,120,132–138} In these studies, leukocytes were identified as a main source of MMPs, the best-studied of which in MS is MMP-9. MMP-9 mRNA, protein, and activity levels are increased in mononuclear blood cells, serum, and CSF, and correlate with barrier leakage and disease progression.^{121,139–143}

Immune cells from the blood can cross the blood–brain barrier via a transcellular (likely no MMPs involved) or a paracellular (MMPs involved) pathway. For the latter, it has been reported that T cells, monocytes, and dendritic cells express and release active MMP-2 and MMP-9, which open the brain endothelial tight junctions to cross the barrier and migrate into the brain.^{118,144–149} After passing the tight junctions, MMP-2 and MMP-9 cleave the transmembrane receptor β -dystroglycan, which anchors astrocytic endfeet to the basement membrane.¹¹⁸ In addition, in lesional MS tissue, MMPs-1, -2, -3, -9, and -19 have been detected in microglial nodules and microglial-like cells where they contribute to inflammation and further destabilize the blood–brain barrier.^{150,151}

One MMP that currently gets attention in the MS field is MMP-12. MMP-12, also called macrophage metalloelastase, is assumed to be essential in the

pathogenesis of MS, most likely due to its primary myelin- or oligodendrocyte-toxic potential and its role in macrophage extravasation. At the same time, MMP-12 does not seem to damage the blood–brain barrier or alter ECM remodeling and deposition.^{138,152} In contrast, data from other studies show that MMP-12 KO mice with EAE had a significantly worse maximum severity and disease burden compared with EAE wild-type control mice, suggesting that increased MMP-12 expression levels are protective in MS.¹²⁷ An additional study showed that wild-type and MMP-12 KO mice with EAE followed a relapsing-remitting course.¹⁵³ Although both mouse groups had a similar clinical onset, relapses in MMP-12 KO mice with EAE were more severe and their residual disability at remission was higher.

Thus, while it is clear that MMPs contribute to MS, it is less clear if this occurs by degrading the endothelial basement membrane, which would facilitate leukocyte extravasation and migration into the brain (Figure 5(a)). In the brain, leukocytes then release more MMPs that contribute to the overall MMP effect of axonal demyelination and neuronal cell death.

Cerebral aneurysms

An aneurysm is a blood-filled balloon-like bulge in the wall of an artery. The causes of brain aneurysms are manifold and include aging, atherosclerosis, hypertension, and severe head injury, all of which are accompanied by neuroinflammation.¹⁵⁴ Most cerebral aneurysms remain undetected until they rupture, which is life-threatening.¹⁵⁵ Therefore, it is critical to prevent rupture by securing intracranial aneurysms, which is accomplished by invasive brain surgery.¹⁵⁶ A less invasive approach would be to prevent aneurysms from forming, which requires understanding of aneurysm pathology. One theory states that MMPs degrade the vascular ECM, thereby contributing to localized ballooning of a blood vessel and leading to aneurysm formation and growth.^{157–159} For example, in human brain samples, protein expression levels of plasmin, MMP-2, MMP-9, and MT1-MMP were increased in the aneurysmal wall compared to normal cerebral arteries and overall MMP-2/MMP-9 proteolytic activity was higher in aneurysm tissue compared to control arteries.¹⁵⁷

Recent reports also indicate that MMPs are involved in vascular calcification,^{160,161} which could be another negative effect MMPs contribute to the pathological outcome of cerebral aneurysms. In a retrospective review, the authors showed that the presence of calcification in an aneurysm was the sole marker of adverse outcome.¹⁶² Larger aneurysms tended to be more likely to be calcified, while size by itself did not have an

adverse effect on outcome. Furthermore, in surgically securing intracranial aneurysms, calcified aneurysms are a significant source of morbidity.¹⁶²

One possibility to limit aneurysm progression and growth is through MMP inhibition, which could potentially reduce the need for invasive treatment.^{163,164} Pre-clinical studies show that MMP inhibitors block aneurysm formation and growth.^{164–166} Xiong et al.¹⁶⁶ demonstrated in a mouse model of Marfan syndrome that inhibiting MMP-2 and MMP-9 protein expression with doxycycline blocked ECM degradation, which significantly delayed aneurysm rupture. Nuki et al.¹⁶⁵ used a mouse model, where 70% of animals develop brain aneurysms and demonstrated that doxycycline reduced the incidence of aneurysms to 10%. The authors also showed a reduced incidence (40%) of intracranial aneurysms in MMP-9 KO mice, whereas over 60% of MMP-2 KO mice still developed cerebral aneurysms, suggesting that MMP-9 is critical for aneurysm formation. Aoki et al.^{167,168} used statins in rats, where cerebral aneurysms were induced by unilateral ligation of the common carotid artery and hypertension. Statin treatment decreased aneurysm size by 30–40% within one month likely through a mechanism that lowered MMP levels, which is thought to delay aneurysm formation and growth.¹⁶⁴ The therapeutic benefit of statins is attributed to their cholesterol-lowering, anti-inflammatory, and anti-NF- κ B effects, which lower MMP activity.¹⁶⁴ Statins have also been studied in humans. Yoshimura et al.¹⁶⁹ conducted a retrospective study analyzing data from 117 patients with ruptured brain aneurysms and 304 patients with unruptured brain aneurysms to assess if statins prevent rupture. In this study, 9% of patients with ruptured cerebral aneurysms used statins, whereas 26% of patients with unruptured aneurysms used statins, which indicates that statins lower the risk of brain aneurysms to rupture.

Together, MMPs contribute to formation, growth, and rupture of cerebral aneurysms by digesting the ECM, which leads to ballooning of blood vessels. This suggests that inhibition of MMPs, especially MMP-9, could potentially prevent cerebral aneurysms.

Stroke

In 2012, stroke accounted for about seven million deaths worldwide, which is 12% of all deaths and makes stroke the number two killer.¹⁷⁰ With an additional 10 million people surviving a stroke each year, over 30 million people altogether survived a previous stroke.¹⁷¹ Stroke is characterized by a loss in brain function due to limited cerebral blood flow (ischemic) caused by a blocked blood vessel, or due to bleeding into the brain parenchyma or subarachnoid space (hemorrhagic).

In stroke, MMPs have detrimental effects in the acute phase and beneficial effects in the post-stroke phase.¹⁷² Detrimental effects are mediated by dysregulated MMPs and include neurovascular disruption and brain parenchymal damage (Figure 6(a)). Various studies in human and rat brains show that protein and activity levels of MMPs-2, -3, and -9 are increased after stroke and MCAO compared to control tissue.^{173,174} These changes in MMP protein and activity levels result in aberrant proteolysis that contributes to blood–brain barrier dysfunction and in part determines the extent of the infarct.^{132,173–177} In addition, studies using rat stroke models suggest that by degrading the basal lamina, MMPs predispose brain capillaries to rupture and hemorrhagic transformations after stroke.^{178–180} Other examples of detrimental MMP effects in stroke were shown in studies using rodent models of focal cerebral ischemia. In these studies, increased MMP-9 protein levels were detected in the acute phase (2–24 h) after stroke that coincided with an opening of the blood–brain barrier. In contrast, MMP-2 protein levels were increased several days after stroke,^{181,182} during which barrier leakage is presumably restored.

While most MMP research in stroke is focused on MMP-2 and MMP-9,^{183,184} other MMPs also have detrimental effects. Suzuki et al.¹⁸⁵ treated mice after thrombotic MCAO with tissue-type plasminogen activator (tPA) and observed an increased incidence of intra-cranial bleeding compared to mice not treated with tPA. The authors showed increased MMP-3 mRNA and protein levels in the capillary endothelium in the infarct region of tPA-treated mice compared to tPA-untreated control mice. Suzuki et al.^{185,186} concluded that in tPA-treated mice, MMP-3 digests the neurovascular basal lamina, thereby opening the endothelial barrier and contributing to intra-cranial bleeding. These findings suggest that MMP-3 has detrimental effects during tPA treatment or is induced by tPA, which is unfortunate since tPA is currently the only FDA-approved treatment for ischemic stroke.

However, MMPs also have beneficial effects during the recovery phase after stroke (Figure 6(b)).¹⁸⁷ Studies suggest that MMP-9, MMP-2, and MMP-7 remodel lesional ischemic and infarct tissue and participate in angiogenesis, vasculogenesis, and neurogenesis.^{17,83,94,188–190} Two mechanisms have been identified by which MMPs exert these effects. First, during tissue remodeling in the post-stroke recovery and healing phase, MMPs digest old ECM so that new ECM and tissue can be generated.^{2,187,191} Second, during ECM digestion, MMPs (mainly MMP-7 and MMP-9, but also MMPs-1, -2, -3, -10, and -11) increase the availability of growth factors (e.g., nerve growth factor,

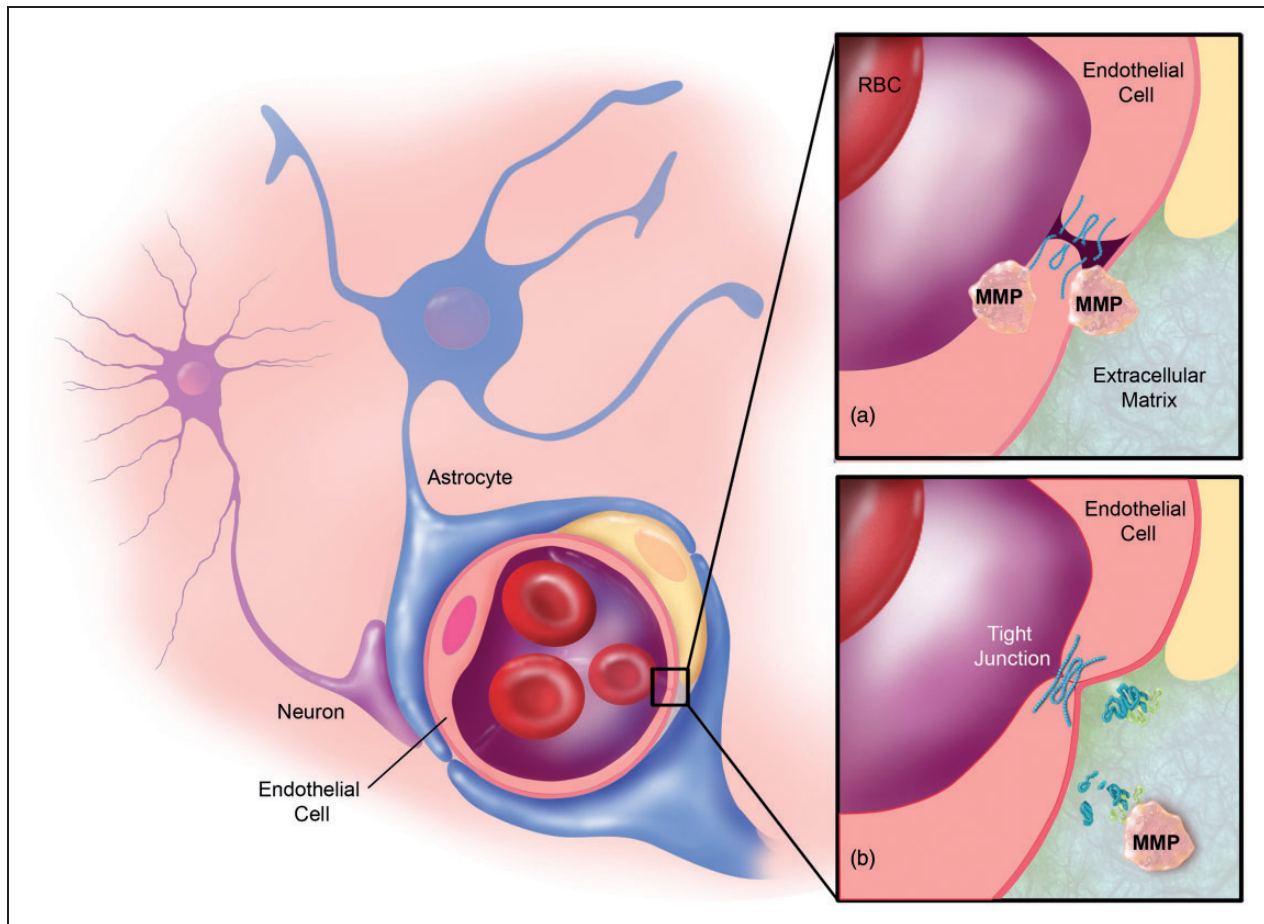


Figure 6. MMP-9 in stroke. (a) acute phase: endothelial cells and recruited leukocytes secrete MMP-9, which degrades the blood–brain barrier, the neurovascular basement membrane, and the ECM. (b) remodeling phase: astrocytes and neurons secrete MMP-9, which contributes to remodeling of the ECM in the neurovascular unit.

brain-derived neurotrophic factor, neurotrophin-3/-4, and vascular endothelial growth factor). This occurs through cleaving inactive growth factor precursors into their active form or through releasing active growth factors by ECM proteolysis.^{54,188} Increased levels of growth factors support tissue remodeling by stimulating angiogenesis, vasculogenesis, and neurogenesis, all of which are critical in stroke recovery. These findings suggest that during the remodeling and healing process, MMPs are involved in the migration of neuronal precursor cells to areas damaged by stroke.^{17,83}

Together, MMPs are essential in stroke in both the acute phase and the post-stroke phase (Figure 6). In the acute phase, MMPs impair barrier integrity and damage the parenchymal tissue, whereas in the post-stroke phase MMPs contribute to the recovery process by remodeling lesional ischemic and infarct tissue and by participating in angiogenesis, vasculogenesis, and neurogenesis.

Epilepsy

The World Health Organization estimates that at least 65 million people worldwide suffer from epilepsy.^{192–194} Epilepsy describes various diseases and seizure syndromes, and patients are diagnosed with epilepsy after recurring, unprovoked seizures.¹⁹⁵

The role MMPs have in epilepsy is still unclear, but studies suggest MMPs contribute to epileptogenesis, epilepsy progression, and brain remodeling after seizures. For example, MMP-9 KO mice are less sensitive to chemically-induced seizures compared to wild-type mice, and conversely, human MMP-9-overexpressing rats are more sensitive to chemical seizure induction, suggesting MMP-9 affects epileptogenesis and/or seizure genesis.^{196,197}

MMP levels are increased in the epileptic brain. In chemically induced animal seizure models and patients with temporal lobe epilepsy, MMP-9 protein and activity levels are increased in neurons of the parietal and

frontal cortex, as well as the thalamus, the regions where the seizures originated.^{73,197–200} Li et al.²⁰¹ detected increased MMP-9 protein and activity levels in CSF from adult epilepsy patients with generalized tonic-clonic seizures compared to age-matched non-epileptic individuals. MMP-9 levels were also increased in serum from patients after seizures. Suenaga et al.²⁰² detected threefold higher MMP-9 protein levels in serum from children with encephalopathy after prolonged febrile seizures compared to serum from healthy children. The authors also found higher serum MMP-9 levels in children with prolonged febrile seizures but no encephalopathy, in children with simple febrile seizures, and in children with convulsive status epilepticus compared to healthy children.²⁰² Research suggests that increased MMP-9 protein and activity levels mainly serve two functions: first, MMP-9 contributes to seizure-induced neuronal cell death; and second, MMP-9 is critical in remodeling neuronal networks after seizures. Jourquin et al.⁷⁵ and Hoehna et al.⁷³ demonstrated neuronal cell death in areas with increased MMP-9 levels after seizures and showed that inhibiting MMP-9 reduced cell death. Other studies show that MMP-9 is involved in structural remodeling, mossy fiber sprouting, diminished seizure-induced pruning of dendritic spines, and decreased aberrant synaptogenesis.^{197,199,203}

MMP-2 function in the epileptic brain is less understood than that of MMP-9. Jourquin et al.⁷⁵ demonstrated that MMP-2 does not contribute to neuronal cell death in epilepsy. However, it is conceivable that MMP-2 contributes to structural remodeling in epileptogenesis since MMP-2 mRNA, protein, and activity are increased after seizures.^{197,199,204}

Several studies demonstrate blood-brain barrier dysfunction in epilepsy and seizure-induced barrier leakage.^{205–210} Additionally, barrier leakage itself triggers seizures, suggesting a pernicious feedback loop contributing to epilepsy progression.^{210–212} In this regard, MMPs most likely degrade tight junction and ECM proteins, which potentially contribute to barrier leakage after seizures.^{98–100} Li et al.²⁰¹ showed that increased MMP-9 protein and activity levels in serum and CSF of patients with generalized tonic-clonic seizures correlate with barrier leakage. Furthermore, barrier leakage is associated with leukocyte extravasation into the brain after seizures. Leukocyte extravasation is a complex, multi-step process that requires MMPs, in particular MMP-2 and MMP-9, both from the endothelium as well as from activated T cells and macrophages.^{188,147,149,213} Li et al.²⁰¹ demonstrated in patients with generalized tonic-clonic seizures that increased CSF leukocyte counts correlated with increased MMP-9 levels in CSF and the degree of barrier leakage.²⁰¹

Thus, MMP-2 and MMP-9 seem to contribute to seizure- and/or epileptogenesis, neuronal network remodeling, neuronal cell death, and barrier leakage after seizures. Little is known about other MMPs in epilepsy.

Alzheimer's disease

AD is a neurodegenerative disorder that affects more than 20 million patients globally.^{214,215} Disease projections are grim and predict up to 100 million AD patients by 2050.²¹⁶ Despite all research efforts, AD etiology and progression are not well-understood and a cure or prevention is currently not available. AD pathology is characterized by brain accumulation of amyloid- β (A β), development of A β plaques, formation of neurofibrillary tangles, and neuroinflammation, all of which contribute to neurodegeneration.^{217,218} In this section, we first describe what is currently known about MMPs in AD and then describe the relationship between A β and MMPs.

Several groups have reported that MMP levels in rodent AD models are increased compared to control animals. Yan et al.²¹⁹ detected increased MMP-9 protein levels in brain slices from transgenic APPsw and amyloid precursor protein (APP)/PS1 mice compared to wild-type mice. Using 5xFAD mice, Py et al.²²⁰ found increased MMP-2, MMP-9, and MT1-MMP protein levels in the hippocampus compared to control mice. MMP-2 and MMP-9 were primarily expressed in astrocytes; MT1-MMP was localized to neurons; MMP-9 and MT1-MMP were also detected in amyloid plaques. Consistent with these findings, several groups found overexpression of MMP-2, -3, and -9 mRNA and protein in postmortem brains from AD patients.^{221–224} Horstmann et al.²²⁵ used zymography and detected MMP-2, -3, -9, and -10 activity levels in serum and CSF from AD patients and compared them to gender- and age-matched healthy control individuals. The authors found that MMP-3 activity was elevated by 40% in plasma and 60% in CSF from AD patients compared to control individuals. MMP-2 activity in the CSF of AD patients was decreased by 32% compared to CSF samples from healthy individuals, while activity levels in plasma remained unchanged. MMP-9 and MMP-10 activity were undetectable in CSF, MMP-10 activity was unchanged in plasma, and MMP-9 activity in plasma was decreased by 41% compared to healthy individuals. Lorenzl et al.²²⁶ detected higher levels of proMMP-9 protein in plasma samples from AD patients compared to control individuals.

Some research aimed at clarifying the relationship between MMPs and A β . Deb and Gottschalk²²⁷ observed in rat hippocampal and astrocyte cultures

that A β ₄₀ induced protein expression and proteolytic activity of MMPs-2, -3, and -9. We demonstrated that exposing isolated rat brain capillaries to A β ₄₀ *ex vivo* increased MMP-2 and MMP-9 protein and activity levels.²²⁸ We made similar observations in a transgenic mouse AD model (Tg2567 hAPP mice), where MMP-2 and MMP-9 levels in brain capillaries were elevated compared to capillaries from wild-type mice. Yin et al.²²⁹ observed in APP/PS1 mice that astrocytes surrounding amyloid plaques secrete more MMP-2 and MMP-9. They also demonstrated that breeding APP/PS1 mice with MMP-2 or MMP-9 KO mice, or pharmacologically inhibiting MMP-2 or MMP-9 in APPsw mice increased A β brain levels by 1.5-fold compared to controls and increased A β half-life by about 50%.²²⁹ Additionally, Yin et al. observed in the phosphate buffer-soluble fraction of cortex and hippocampus of MMP-2 KO mice increased murine A β ₄₀ and A β ₄₂ compared to age-matched wild-type mice, whereas A β ₄₀ and A β ₄₂ were unchanged in the phosphate buffer-insoluble fraction. In the cortex and hippocampus of MMP-9 KO mice, murine A β ₄₂ levels were increased in the phosphate buffer-soluble fraction of hippocampus and cerebral cortex compared to age-matched wild-type mice, while they remained unchanged in the phosphate buffer-insoluble fraction. These effects were due to decreased A β proteolysis and not to increased A β production. These findings suggest that MMPs potentially contribute to A β clearance. In this regard, MMPs-2, -3, and -9 proteolytically degrade A β .^{219,230,231,232} Ridnour et al.²³³ showed that levels of A β ₁₋₁₆, a product of A β metabolism by MMP-9, and MMP-9 activity were decreased in brain lysates of hAPPSwDI mice lacking nitric oxide synthase compared to their littermates expressing nitric oxide synthase. Based on these data, the authors concluded that nitric oxide is potentially involved in clearing plaques through increasing MMP-9 activity. Yan et al.²¹⁹ demonstrated in brain slices of APP/PS1 mice *in situ* that MMP-9 digests both fibrillary A β ₄₂ as well as compact amyloid plaques. In another study, Wang et al.²³⁴ examined *in vivo* the relationship between MMP-9 protein expression and A β plaques by deleting the MMP-9 gene in APP/PS1 mice. In the cortex and hippocampus of these APP/PS1 MMP-9 KO mice, A β plaques were larger in size and number compared to APP/PS1 mice with functional MMP-9. Finally, Liao et al.²³⁵ demonstrated that MT1-MMP degrades both soluble and fibrillary A β peptide in a time-dependent manner *in vitro* and this effect is inhibited by the MMP inhibitors GM6001 and TIMP-2. The authors also showed that MT1-MMP degrades brain fibrillary amyloid plaques in another AD mouse model (hAPPSwDI mice) *in situ*.

These findings suggest an inverse relationship between MMP-2/MMP-9 and A β , where one would expect low MMP levels in the AD brain with high A β load. However, MMPs are upregulated in the AD brain, which is counterintuitive considering the above-mentioned findings. One explanation for this could be that MMP-mediated A β degradation is too low to prevent A β brain accumulation. While MMPs might be involved in processing A β and clearing plaques, they are likely not major players in this process.^{219,229}

Thus, MMPs are increased in the AD brain, but the role of MMPs in AD is unknown. The current literature is unclear on whether changes in MMP levels contribute to AD progression or might have beneficial effects on the disease. While it is possible that MMPs play no major role in AD, studies show that MMPs could potentially be involved in processing A β and AD progression.

Parkinson's disease

PD is a neurodegenerative movement disorder with more than six million patients worldwide.²³⁶ PD was first described by James Parkinson²³⁷ in 1817, but to this day, many aspects of the disease are unknown. At the molecular level, PD is characterized by accumulation of α -synuclein in dopaminergic neurons, resulting in the formation of Lewy bodies, cell damage, and neuronal death of dopaminergic neurons. PD is also accompanied by neuroinflammation aggravating the disease.^{238,239}

In recent years, MMPs have received some attention in the PD field. Lorenzl et al.²⁴⁰ determined protein expression and activity levels of MMPs-1, -2, and -9 in postmortem brain tissue from PD patients and age-matched control individuals. While the authors observed no change in MMP-1 and MMP-9, they detected a 50% reduction in MMP-2 activity levels in the substantia nigra of PD patients compared to control individuals. The authors suggested reduced MMP-2 activity could help neurite outgrowth of surviving dopaminergic neurons in the substantia nigra.²⁴⁰

In addition to MMPs-1, -2, and -9, PD research has focused mainly on MMP-3. Three mechanisms have been suggested on how MMP-3 could be involved in PD. First, using *in vitro* cell lines and primary cultures of dopaminergic neurons from rat, Choi et al.²⁴¹ observed that active MMP-3 is released from apoptotic dopaminergic neurons and that MMP-3 protein levels are higher compared to healthy, non-apoptotic dopaminergic neurons. Using the MTPT mouse PD model, Chung et al.²³⁸ found increased MMP-3 protein and activity levels compared to control mice resulting in apoptosis and cell death. MMP-3 was also involved in

caspase-3 activation, specifically in apoptotic signaling upstream of caspase-3 and downstream of c-Jun N-terminal kinases.^{238,241–244}

Second, MMP-3 might potentially be involved in α -synuclein cleavage. Sung et al.²⁴⁵ showed that MMP-3 cleaves purified α -synuclein *in vitro* and that α -synuclein aggregation increased in the presence of MMP-3-cleaved α -synuclein fragments compared to a solution without these fragments. Furthermore, aggregates of α -synuclein fragments were more toxic in cell viability assays compared to aggregates of non-fragmented α -synuclein. Sung et al.²⁴⁵ also demonstrated that MMPs-1, -2, -9, and MT1-MMP cleave purified α -synuclein as well, however, they were less effective than MMP-3. Moreover, Kim et al.²⁴⁶ showed in microglia cultures and in a 6-OHDA mouse PD model that α -synuclein-induced MT1-MMP expression supports cell migration of reactive microglia into the pathological region, which accelerated PD pathogenesis.

Third, recent data indicate involvement of neuroinflammatory events such as microglial activation, T-leukocyte infiltration, and blood–brain barrier dysfunction in PD.^{238,247–249} This is consistent with data from Chung et al.,²³⁸ who showed infiltration of peripheral immune cells and brain uptake of FITC-albumin (70 kDa) in the MPTP mouse PD model, indicating neuroinflammation and barrier leakage. The authors showed in MMP-3 KO mice that barrier leakage was attenuated and the number of immune cells infiltrating the substantia nigra was decreased, demonstrating MMP-3 involvement in the MPTP mouse model.

In conclusion, MMP-3 seems to be involved in dopaminergic neurodegeneration, neuroinflammation, and barrier leakage in PD. More research will have to clarify the role MMPs play in PD and if MMP inhibition could be a valid therapeutic strategy.

Brain cancer

In 2012, more than 250,000 people were newly diagnosed with brain cancer and nearly 190,000 patients died worldwide.^{250,251} Survival rates for adult brain cancer patients are low. Even with aggressive therapy, median survival of patients with glioblastoma multiforme, the most common and deadliest malignant brain cancer, is only 12–17 months.^{252–254} Thus, to effectively treat brain cancer, new approaches and interventions are desperately needed.

MMPs are potential therapeutic targets in cancer because they play a role in cancer biology. In this regard, mRNA and protein overexpression of MMPs-1, -2, -3, -7, -8, -9, -13, and MT1-MMP has been

observed in many malignant peripheral and CNS tumors, and a correlation between MMP expression, tumor aggressiveness, tumor stage, and prognosis has been demonstrated.^{255,256} Indeed, secreted MMPs (MMPs-1, -2, -3, 7-, -8, -9, -10, -11, -13) as well as membrane-bound MT-MMPs are critical for the formation of cancer metastases, their invasion into the brain, and the formation of secondary tumors, and MMPs participate in most of the steps of the metastasis process (Figure 7).^{84,92}

1. Formation of metastatic cells at the primary tumor: Li et al.²⁵⁶ showed in primary tumors that MMP-7 converts the transmembrane cell–cell adhesion protein E-cadherin into a soluble protein resulting in ineffective binding between tumor cells, which allows cancer cells to split from the primary tumor and form metastases that can enter the bloodstream.
2. Intravasation of metastatic cells into the blood circulation: Juncker-Jenson et al.²⁵⁷ found that MMP-1 participates in the intravasation of metastatic cells from a human HEP3 epidermoid carcinoma graft in chick embryos. The authors showed that MMP-1 regulating endothelial permeability and transendothelial migration supported tumor invasion by activating the endothelial non-tumor/non-matrix receptor PAR1. The authors also used grafts with naturally acquired or experimentally induced MMP-1 deficiency and found that intravasation was decreased by more than 80%.²⁵⁷
3. Metastatic cell adhesion to the brain capillary endothelium: it is unclear if MMPs participate in this step of the metastasis process but given the various functions MMPs have it is conceivable. Hummel et al.²⁵⁸ showed that MMPs-2, -3, -9, and -12 are involved in the shedding of cell adhesion molecules (e.g., vascular cell adhesion molecule-1, intercellular cell adhesion molecule-1) from the plasma membrane of human brain endothelial cell cultures after TNF- α -induced MMP upregulation. MMPs might also contribute to shedding CD44 in metastatic cell adhesion to the capillary endothelium.²⁵⁹ This is particularly interesting since CD44 is a cell-surface glycoprotein in endothelial cells, leukocytes, and many metastatic cancer cells, where it presents selectins thereby facilitating adhesion of the host cell to the brain capillary endothelium.^{260–262}
4. Metastatic cell extravasation: MMPs contribute to metastatic cell extravasation and facilitate paracellular transmigration of tumor cells across brain capillary endothelial cells *in vitro* and the blood–brain barrier *in vivo*.^{183,263–265} Lee et al.²⁶⁴ showed that MMP-2 contributes to the migration of breast cancer cells across the cell monolayer of an *in vitro*

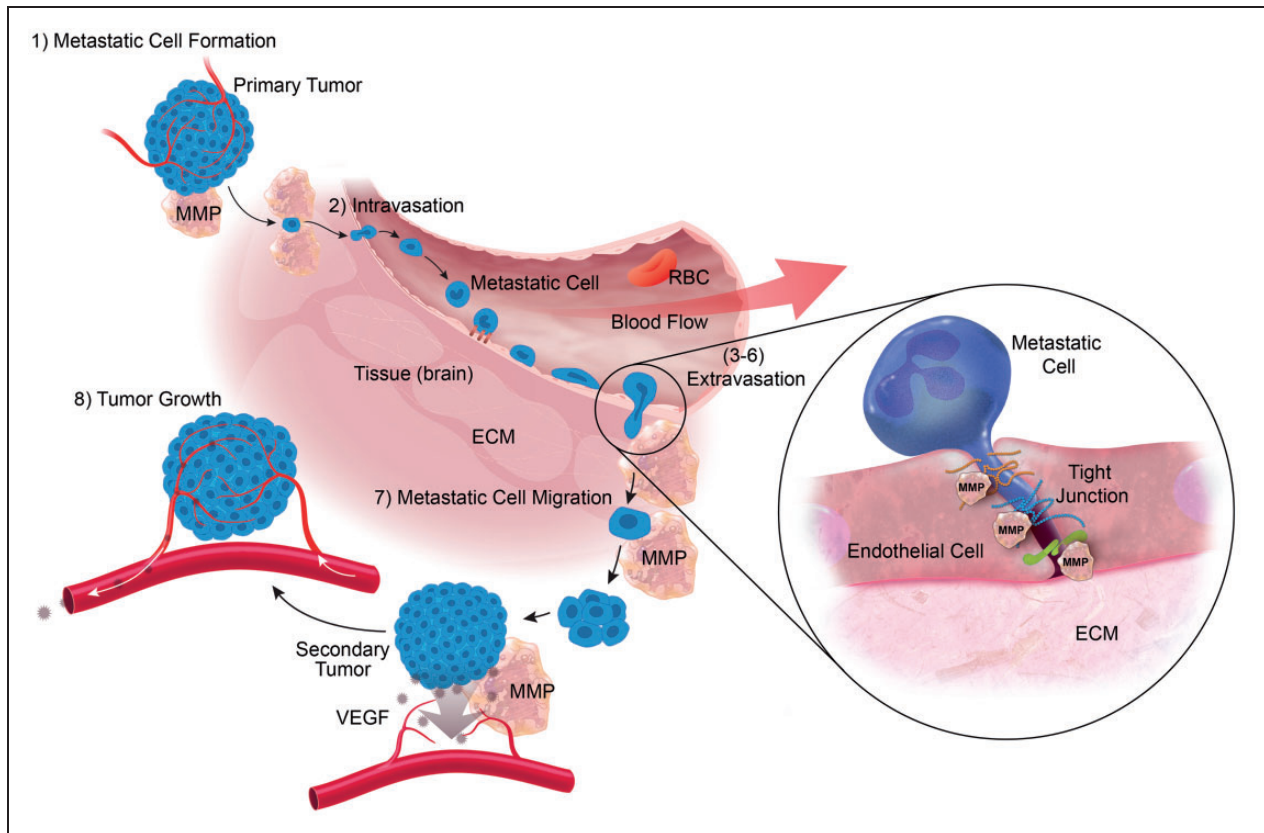


Figure 7. MMPs in cancer metastasis. MMPs participate in most steps of the cancer metastasis process. (1) Formation of metastatic cells at the primary tumor. (2) Intravasation of metastatic cells into the blood circulation. (3–6) Extravasation of metastatic cells across the blood–brain barrier into the brain. (7) Tumor cell migration in the brain. (8) MMPs contribute to the tumor microenvironment and tumor angiogenesis.

human blood–brain barrier model. Lörger and Felding-Habermann²⁶⁵ demonstrated *in vivo* that breast cancer cells injected into the left internal carotid artery of BALB/c mice resulted in brain metastases. For paracellular extravasation, endothelial cell–cell contacts have to be loosened so that metastatic cells can migrate across the endothelium. This requires junction proteins to be degraded. Indeed, MMPs proteolyze tight junction and adherence junction proteins, thereby opening the paracellular route.^{99,100,255,263} Feng et al.⁹⁹ demonstrated in leukemic BALB/c nu/nu mice that leukemic cells secrete MMP-2 and MMP-9, which degraded the tight junction proteins zonula occludens-1, claudin-5, and occludin. Thus, MMPs are critical for leukemic and other cancer cells to cross the capillary endothelium and access the brain.

5. Metastatic cell adhesion to the ECM: several membrane-type MMPs (MT1-, 2-, 3-, 5-MMP) shed the cell-surface glycoprotein CD44, which is critical in metastatic cell adhesion to the luminal endothelial membrane and the ECM on the basolateral side of

the endothelium.^{259,266–268} CD44 is involved in presenting cytokines, chemokines, cell adhesion molecules, growth factors, and other proteins such as MMP-2 and MMP-9 to receptors on metastatic and endothelial cells and mediates signaling that regulates metastatic cell migration and invasion.^{267,269} CD44 also interacts with ECM proteins like fibronectin, thereby supporting metastatic cell adhesion to the ECM.²⁷⁰

6. MMPs in ECM proteolysis: localized opening of the ECM (Figure 7) is necessary for metastatic cells to bypass it and MMP-2 and MMP-9 seem to support this process through ECM proteolysis. Wang et al.²⁷¹ compared MMP-2 and MMP-9 levels in human glioma samples with those from control individuals and showed that MMP-2 and MMP-9 levels in gliomas were increased and correlated with the degree of glioma malignancy. The authors also showed that MMP-2 and MMP-9 staining in gliomas was localized to the cytoplasm of tumor cells, endothelial cells, and their ECM. They concluded that by degrading the ECM, MMP-2 and

MMP-9 are determining factors of glioma invasiveness and angiogenesis.²⁷¹ Other studies in cancer cells showed that CD44 captures MMP-2 and MMP-9 at the tumor cell surface, where MMPs then locally digest the ECM surrounding the tumor cells during extravasation.^{272,273}

Other MMPs might also contribute to ECM proteolysis. While conclusive *in vivo* proof in support of this is still missing, there are many findings that point in this direction. Shiomi et al.²⁷⁶ reported increased MT1-MMP mRNA and protein levels in resected glioblastoma tissue compared to non-tumor control tissue. MT1-MMP levels correlated with pro-MMP-2 activation and tumor malignancy and the authors speculated that MMP-2 and MT1-MMP likely contribute to glioma invasion through degrading brain ECM proteoglycans and glia limitans. Nakada et al.²⁷⁴ observed increased MT1-MMP and MT2-MMP mRNA and protein levels in astrocytomas compared to control brain tissue and concluded that both activate MMP-2, which then degrades the ECM. Other groups detected that MT3-MMP directly cleaves ECM components such as type III collagen, proteoglycan, and interstitial collagens.^{275,276} These findings are key for cancer metastasis and invasion since they result in ECM digestion.

7. Metastatic cell migration: for a secondary tumor to form, cancer cells require space when settling in new tissues. This space is likely generated through MMP-mediated ECM degradation and remodeling. In this context, Belien et al.²⁷⁷ showed MT1-MMP digesting axonal myelin proteins that inhibit cell migration and neurite outgrowth. Given that invasive glioma cells preferentially migrate along white matter fiber tracts and that MT1-MMP degrades cell migration-inhibiting proteins that are embedded within white matter fiber tracts, these observations suggest that MT1-MMP facilitates cancer cell migration, thereby increasing glioma malignancy.
8. MMPs in the tumor microenvironment and tumor angiogenesis: increasing evidence suggests MMPs establish and maintain a microenvironment that supports tumor survival and growth. MMPs facilitate cancer cell proliferation by regulating cytokines, growth factors, and cell adhesion molecules that attract tumor cells and support metastatic cell spreading.²⁵⁵ Thus, increased MMP levels are considered to correlate with increased tumor malignancy, and studies report that MMP mRNA, protein, and activity levels are increased in cancer.^{255,256} Xu et al.²⁷⁸ observed increased MMP-1 protein levels in gliomas compared to levels in resected brain tissue from epilepsy patients. Levels of MMPs-1, -2, -3, -7, -8, -9, -13, MT1-, 2-, 3-, 5-,

and MT6-MMP were also increased in brain tumors compared to non-cancerous brain tissue.^{253,274,279,280}

The same MT-MMPs activate proMMP-2 and proMMP-13, and activity and protein expression levels of those MT-MMPs correlate with pro-MMP-2 activation in gliomas, and thus, with tumor malignancy.^{256,279,280}

MMPs also contribute to angiogenesis, which is critical for the tumor microenvironment because blood vessels supply tumors with oxygen and nutrients, thereby supporting tumor survival, growth, and increasing tumor malignancy.^{58,110,281} Angiogenesis is based on endothelial cell migration into surrounding connective tissues and MMPs are critical in this process.^{282,283} MMPs degrade the ECM, release ECM-sequestered pro-angiogenic compounds such as vascular endothelial growth factor, process growth factors, integrins, and adhesion molecules, thereby balancing pro- and anti-angiogenesis.^{58,110,281} Tumor-induced angiogenesis is important to sustain growth of solid tumors and the functional roles of MMPs in tumor angiogenesis are well established.^{85,93} For example, MMPs help recruit pericytes, which are localized in tumor blood vessels and are critical for the development of a functional vascular network. MMPs participate in several steps of the pericyte recruitment process. First, MMPs degrade the ECM to allow pericyte invasion. Second, MMPs stimulate pericyte proliferation and protect pericytes from apoptosis. Third, MMPs help recruit bone marrow-derived stem cells, which differentiate into pericytes.⁸⁶

Angiogenesis is essential for tumor growth and blocking angiogenesis is considered a valid strategy to control malignant tumors. Thus, MMPs can be beneficial in cancer due to their anti-angiogenic effect that is based on processing growth factors, integrins, and adhesion molecules. For example, tumor angiogenesis is reduced in integrin- $\alpha 1$ -null mice compared to wild-type mice.⁸⁷ Integrin- $\alpha 1$ -null mice overexpress MMP-9, which cleaves angiotatin from plasminogen, and angiotatin inhibits endothelial cell growth, resulting in tumor growth inhibition.⁸⁷

MMPs also influence the tumor microenvironment by increasing the permeability of the vascular endothelium in brain tumors, which is then referred to as "blood-tumor barrier". Thus, the blood-tumor barrier is leaky compared to the healthy, intact blood-brain barrier, which helps supply the tumor with an increasing demand of nutrients.^{284,285} Noell et al.²⁸⁶ showed increased MMP-2, -3, and -9 immunoreactivity in brain slices of human primary glioblastomas compared to non-tumor brain tissue and assumed blood-brain barrier leakage in this area. Studies

from other groups detected increased proMMP-2 and proMMP-9 levels in the CSF of dogs with intracranial tumors compared to healthy dogs.^{287,288} Turba et al.²⁸⁸ attributed increased CSF proMMP-9 levels to the recruitment of leukocytes by the tumors and speculated that MMP-9 likely facilitated leukocytes bypassing the blood–brain barrier in order to reach the tumor, and that those leukocytes secreted MMP-9 into the CSF.

In conclusion, MMPs are critical in many aspects of brain cancer. Their main role lies in facilitating metastasis and angiogenesis, which makes them interesting targets for brain cancer treatment and prevention of secondary brain tumors.

MMP inhibition as a therapeutic strategy

Pharmacological inhibition of MMPs is a potential therapeutic strategy for the treatment of CNS disorders. MMP inhibitors such as marimastat, batimastat, and doxycycline could potentially be used in patients. Currently, however, the only FDA-approved MMP inhibitor is the tetracycline analogue doxycycline (Periostat®) for the treatment of periodontal disease.^{289–291} The major obstacle hindering the development of MMP inhibitors for clinical use in patients is our lack of knowledge and understanding of the complex MMP biology and the role MMPs play in CNS disorders such as cerebral aneurysm, epilepsy, AD, and PD. Nonetheless, there is a wealth of preclinical data that support MMP inhibition as a treatment strategy in MS, stroke, and brain cancer.

First, a number of MMP inhibitors decrease the incidence and severity of EAE in animal MS models.^{145,292–295} The MMP inhibitor Ro31-9730 suppressed EAE in rats,²⁹⁵ and minocycline reduced MMP-9 protein and activity in T cells and suppressed EAE in mice.²⁹² Second, preclinical data from various mouse and rat cancer models including colon and breast cancer showed that batimastat reduced tumor growth, number, and occurrence of secondary lung and lymphatic metastases.^{296–299} Third, MMP inhibition with GM6001 or BB-94 in rodent stroke models shortly (hours) after stroke reduced edema, infarct size, and the number of hemorrhagic events.^{180,185,300,301} Long-term (days) MMP inhibition with the MMP-inhibitor BB-1101 for up to 48 h after stroke in rats reduced barrier leakage, but function in neurologic and behavioral tests did not improve.³⁰² Inhibiting MMPs with FN-439 or BB-94 in a rat stroke model for one week even aggravated ischemic brain injury and seemed to halt functional recovery.¹⁹¹

Even though MMP inhibition seems promising in animal models, this has not or only partially been

demonstrated in clinical studies. In an MS trial, 16 patients with relapsing-remitting MS were treated with a doxycycline/interferon combination for 4 months (NCT00246324³⁰³). Doxycycline/interferon treatment reduced brain lesions, which correlated with reduced MMP-9 serum levels and improved post-treatment EDDS values with only one patient relapsing. Overall, doxycycline/interferon treatment was considered effective, safe, and well-tolerated; it was concluded that a trial with a larger patient cohort should be conducted. However, a report on a follow-up trial has thus far not been published.

In a stroke trial, minocycline was given with/without tPA to 60 patients within 6 h after acute ischemic stroke (NCT00630396³⁰⁴). The mean baseline NIH Stroke Scale score was 8.5 ± 5.8 (moderate stroke). Minocycline did not cause severe hemorrhages in tPA-treated patients, was concluded to be safe and well-tolerated up to 10 mg/kg, i.v. alone and in combination with tPA, and was considered ideal for a tPA combination therapy. In another clinical study, Lampl et al.³⁰⁵ showed that minocycline significantly improved patient outcomes. Specifically, NIH Stroke Scale and Rankin Scale scores were significantly lowered, and the Barthel Index was significantly increased. Moreover, participants are currently being recruited for a study testing the safety and efficacy of minocycline in acute cerebral hemorrhage (MACH trial; NCT01805895).

Lastly, several clinical studies testing MMP inhibition in brain cancer have been conducted. In two phase-II trials, a combination of marimastat and temozolomide was tested in recurrent GBM and gliomas.^{306,307} These trials showed that marimastat/temozolomide appears to increase progression-free survival (PFS): at six months, PFS was 39% for GBM (target PFS: 10%) and 54% for glioma (target PFS: 40%) compared to temozolomide treatment alone. Other brain cancer trials with MMP inhibitors showed no improvement. Prinomastat/temozolomide compared to temozolomide alone did neither improve the one-year survival rate nor PFS (NCT00004200, Pfizer). Likewise, phase I and II trials with the MMP inhibitor COL-3 in recurrent high-grade gliomas do not warrant further studies (NCT00004147^{308,309}). In addition, clinical trials testing more than 50 MMP inhibitors for the treatment of cancer have all failed.^{310–322} Vandenbrouke and Libert³¹⁹ summarized the reasons for failure, which included the complexity of MMP biology and lack of MMP knowledge. The authors also listed suboptimal trial design, inadequate clinical endpoints, use of metabolically unstable MMP inhibitors, poor oral bioavailability, no effect, toxic adverse effects, and discrepancies between pre-clinical animal models and human patients.³¹⁹

In summary, while much effort has gone into MMP research over the decades, the field is still far away from

a therapeutic breakthrough. Thus, more work remains to be done to test if MMP inhibition is a viable therapeutic strategy.

Conclusion and future perspectives

Here, we review and summarize current knowledge and understanding of the role MMPs play in health and disease in the brain, particularly the blood–brain barrier. While we know that MMPs participate in important neurophysiological functions and have basic understanding of their role in pathological conditions such as neuroinflammation, MS, stroke, and brain cancer, we know little about MMPs in other CNS disorders such as cerebral aneurysms, epilepsy, AD, and PD.

The picture that emerges is complex: a large number of diverse MMPs covers a broad spectrum of different functions in various physiological and pathophysiological processes resulting in a wide range of effects with varying impact. These MMP effects can be both beneficial and detrimental in the same disease depending on location, time point, and other factors. Thus, MMP expression and functional activity vary significantly and are context-dependent. However, one pattern all the diseases mentioned here have in common is neuroinflammation that involves MMPs.

From what we know today, MMP inhibition as an (additional) therapeutic option is problematic and has a history of failure, but is still discussed for the treatment of CNS disorders. Considering the knowledge gap in MMPs, more research needs to be done while avoiding the mistakes of the past. Specifically, research mainly focused on MMP-2 and MMP-9 needs to be expanded to other MMPs to understand the role each MMP plays in health and disease and to gain better insight into MMP biology in general. For example, elucidating the mechanisms responsible for MMP regulation could provide new opportunities for therapeutic intervention. In addition, specific and selective MMP inhibitors that can be used safely with no or only minor adverse effects need to be identified.

Clearly, we are only beginning to understand MMP biology in the larger context of health and disease. In the future, it will be critical to assess if MMPs can be attractive therapeutic targets to advance the treatment of neurodegenerative diseases.

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Author's contributions

RGR and BB wrote the manuscript; AMSH contributed to the content of the manuscript and critically revised the text, tables and figures for scientific content.

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